Standard Operating Procedures for Magnetite Nanoparticles Synthesis.

Read these instructions completely and thoroughly before attempting the procedure.(See the MSDS sheets for safe handling of the chemicals)

Process: Templated co-precipitation synthesis of magnetite nanometer-sized crystals in gels

Materials and small equipment required:

- Lab Notebook, absolutely must.
- Vacuum pump, sub micron vacuum gauge, cold vapor trap filled with 2-propanol and dry ice;
- 25% (w/w) Pluronic F127 gel, prepared in advance and stored in the fridge;
- Buffer solution, protein solution, peptide solution stored in the fridge;
- Small (5 mL, 10 mL, and 25 mL) roundbottom and pear-shaped flasks with rubber septa and cable ties, cork rings for the flasks;
- Disposable test tubes with rubber septa and cable ties; test tube rack;
- Two-line vacuum manifold with stopcocks equipped with a gas bubbler;
- Vacuum grease;
- Two 250 mL roundbottom flask with an arm equipped with magnetic stir bar, and rubber septum. One is used for degassing the water, the other for degassing of 1M NaOH solution.
- Compressed argon tank equipped with a pressure regulator, gas line and drying insertion;
- Automatic pipettor suitable for drawing 20 μL, 100 μL and 1000 μL solutions.
- 20 lb dry ice Styrofoam container with lid. Work in the fume hood and use nalgene beakers when handling dry ice. Use caution when adding (slowly!) dry ice to the cold trap dewar.

Procedure

All preparations are to be done in the fume hood. Prepare all the stock solutions (25% (w/w) F-127 Pluronic gel, 1M NaOH, 0.33M $FeCl_2$ and 0.66M $FeCl_3$) in advance, before you start synthesis. Make sure ALL liquids are degassed an /or sparged with argon, and all flasks are filled with argon. Note any color change or cloudiness of solutions, and filter them as needed prior to use.

- All synthesis and washing procedures are to be conducted in the fume hood.
- Take caution in operating the vacuum pump, make sure the condenser is filled with iso-propanol /dry ice mixture before turning on the pump.
- In a 250 mL roundbottom flask with an arm equipped with magnetic stir bar, pour 150 mL of nanopurified water. Cap the flask with a rubber septum and secure it with a cupper wire. Degas the water: evacuate the air with vacuum pump for 15 minutes, refill the flask with argon. Repeat three times. Seal the arm of the flask after filled with argon for the third time.
- In a 250 mL roundbottom flask with an arm equipped with magnetic stir bar, weigh enough NaOH pellets to prepare 150 mL of 1M NaOH solution. Cap the flask with a rubber septum and secure it with a cupper wire. Degas the pellets for 10 minutes. Add nanopurified water to prepare 150 mL of 1M NaOH solution. Degas the water: evacuate the air with vacuum pump for 15 minutes, refill the

flask with argon. Repeat three times. Seal the arm of the flask after filled with argon for the third time.

- In 25 mL roundbottom flask, weigh enough $FeCl_2 \times 4H_2O$ to prepare 20 mL of 0.33M $FeCl_2$ solution. Cap the flask with a rubber septum and secure it with a small cable tie. Degas the powder for 3 minutes. Add nanopuritied water to prepare 20 mL of 0.33M $FeCl_2$ solution. Sparge the solution with argon for 2 minutes, leave the flask under some argon pressure.
- In 25 mL roundbottom flask, weigh enough $FeCl_3 \times 6H_2O$ to prepare 20 mL of 0.66M $FeCl_3$ solution. Cap the flask with a rubber septum and secure it with a small cable tie. Degas the powder for 3 minutes. Add nanopuritied water to prepare 20 mL of 0.66 M FeC3 solution. Sparge the solution with argon for 2 minutes, leave the flask under some argon pressure.
- In a 50 mL RB flask, weigh Pluronic F127, add water to prepare 25% (w/w) solution, shake well, cap with a rubber septum and place in the fridge. Shake well every two hours to ensure proper dissolution of powder, return to the fridge.
- Procedure is described for synthesis in the test tubes, working with RB or pea-shaped flask is identical. Mark the test tubes. With an automatic pipettor, add 500 μL of cold Pluronic 25% (w/w) solution to the test tube. Add required amount of cold buffer, peptide or protein. Cap the test tube, secure the septum with a small cable tie, and gently swirl the test tube to ensure proper mixing of ingredients. Make sure to copy the list of all the reaction tubes into your lab notebook. Degas the contents of the test tube: evacuate the test tube for 1 minute, refill with argon for 5 seconds. Repeat evacuation and argon-refill three times. Place the rack into the fridge for 10 minutes to ensure equilibration of all reagents.
- Using the disposable 100 μL syringe, inject the test tubes with 50μL of 0.33M $FeCl_2$ solution and 50μL of 0.66M $FeCl_3$ solution. The ratio of Fe(II) to Fe(III) ions has to be maintained as 1:2. Gently swirl the test tube to ensure proper mixing of ingredients. Flush with argon: a) open the argon, b)insert a long needle connected to the vacuum two-line manifold, c) insert a small disposable needle to ensure argon is allowed to escape. Remove both needles. Place the rack into the fridge for 15 minutes to allow proper incubation mixture with iron ions. Avoid contact with iron chloride solutions as they will stain your hands and clothing. These stains are very hard to remove.
- After 15 minutes of incubation time, remove the rack with the test tubes from the fridge and leave at room temperature for 30 minutes to allow gelation of Pluronic F127 solution.
- Fill the 3 mL disposable syringe with 2.7 mL of nanopurified water. Fill the same syringe with 0.3 mL of 1M NaOH solution. Allow formation of a small air bubble and use it to mix the liquids for several seconds. Squeeze the air bubble out of the syringe now filled with 0.1M NaOH.
- Under slow argon flow (use the same two needles again), slowly inject each test tube with needed amount of 0.1M NaOH solution, on top of the gel. Observe formation of a thin dark band at the gel-base interface.
- Leave test tubes in the fume hood at room temperature. Check the propagation of the reaction front daily. After 5-7 days, all remaining gel should darken.

Washing of samples

- Insert the test tube into magnet and slowly pour excess liquid into the glass beaker. Refill the test tube with 3 mL of fresh, argon-filled nanopurified water, shake and let precipitate with magnet. Repeat three times.
- Cap the remaining sample.

TEM sample preparation

- Insert a clean 2 mL disposable Pasteur pipette into the test tube with the sample. Capillary action will force the suspension with nanoparticles to rise in the pipette, therefore no need for a pipette bulb.
- Place your finder on top of the pipette, take the pipette out, and press your finger to dispense one drop of suspension into a clean test tube. Fill the tube with 3 mL of nanopurified water, shake well. Using a clean glass pipette, place one drop of the diluted suspension on a dull side of cupper-supported carbon TEM grid. Let sit for 1 minute, and then gently blot the side of the grid with a filter paper wegde.
- Allow 1 hour to dry. Sample is ready to be examined with TEM.

Laboratory Safety, Hygiene and Etiquette

- Wear proper personal protective equipment while in the lab:
  - Safety glasses with side shields
  - Closed toe shoes
  - Gloves
  - Lab coat
- All work has to be done in the fume hood.
- Lab Notebook – mandatory. Do write down everything you do, no matter how insignificant it may seem.
- Absolutely no food or drink is allowed in the lab, no exceptions.
- Read the MSDS's for all chemicals involved BEFORE synthesis.
- If you are the first person to open the bottle with chemical, label it with your initial and date. Label all your samples and keep a list in the lab notebook.
- Check the vacuum manifold and regrease the stopcocks as needed. Check the mineral oil in the bubbler and replace it if looks dirty.
- Change the vacuum pump oil every three month if the pump is used for degassing of aggressive solvents. Otherwise, change the pump oil every six month. Label the pump with your initials and date of the oil change. Used oil is to be collected only in a plastic jar labeled "used oil", located in room 138 (Spedding).
- Clean up after yourself
  - Clean the hood up after you are done.
  - Disposable pipettes are to be collected in the wide-mouth nalgene jar. Once the jar is filled, transfer its contents to the cardboard "Glass" receptacle located on the floor in room 141 (Spedding). Avoid disposing sharps into the same receptacle.

- Disposable syringes are to be collected in a "Shaprs" receptacle located in the corner of the hood. Use the same sharps receptacle for disposing used razor blades, and cupper wire. Once a receptacle is filled, transfer it to the waste accumulation area hoof, cover the opening and call HIS for pick up. Replace the receptacle with an empty one: this can be either purchased in the stock room, or you can use one from the bench top.
- Do not pour chemicals down the drain. Rinse the glassware with proper solvent and dispose the solvent in the waste bottle. Do write down what and how much is being disposed. Place the used and rinsed glassware into the glassware bin filled with Alconox solution. Leave the glassware to soak for a day or two before washing it.

Authorized User's List/Safety Training Statements

Project: Bio-inspired protein-templated synthesis of magnetite nanoparticles

Location: 141-136 Spedding Hall Ames Lab

As a researcher working on the above project, I

(a) have received training from the primary operator or group leader on the standard operating procedures;

(b) have attended the required Ames Laboratory and Iowa State University Safety Training modules including Hazard Communication (AL-150), Hazardous Waste Generator and Chemical Hygiene Plan;

(c) have read the standard operating procedures documents for this project;

(d) know and understand the information in the items above and feel qualified to operate the systems safely.

User signatures		Date
Tanya Prozorov	To T. Prozorov	1/12/07
Daniel Orr	Daniel Orr	1/12/07
Jorge Almodovar		1/12/07
Surya Mallapragada (Group leader)		1/12/07